Biological Control **20,** 48–56 (2001)
doi:10.1006/bcon.2000.0873, available online at http://www.idealibrary.com on IDEAL®

Control of Codling Moth, *Cydia pomonella* (Lepidoptera: Tortricidae), with *Steinernema carpocapsae:* Effects of Supplemental Wetting and Pupation Site on Infection Rate

Thomas R. Unruh[1] and Lawrence A. Lacey

Yakima Agricultural Research Laboratory, USDA-ARS, 5230 Konnowac Pass Road, Wapato, Washington 98951

Received March 20, 2000; accepted August 7, 2000; published online December 13, 2000

Infection of cocooned codling moth (*cydia pomonella*) larvae by the entomopathogenic nematode *Steinernema carpocapsae* was studied in three field experiments. Factors that varied within or between experiments included method of application, type of substrate containing cocooned larvae, time when nematodes were applied, seasonal effects, and supplemental wetting before or after nematode application. Conventional air-blast sprayer applications of 0.5–5.0 million infective juveniles (IJs)/tree in fall resulted in ca. 30% mortality of larvae in cardboard trap bands, whereas hand-gun application (2 million IJs/tree) produced mortality of ca. 70%. Application in the evening caused higher larval mortality than application in the morning when no supplemental wetting was used after treatments. Morning and evening applications caused equivalent larval mortality when a postwetting treatment was included. In a trial conducted in midsummer, supplemental wetting, either before or after hand-gun application of 1 million IJs/tree, enhanced nematode-produced mortality. Mortality approached 100% if both pre- and postwetting was used. Larvae in exposed cocoons on apple wood were infected at a higher rate (86%) than those on wood in less exposed positions (73%) or in nonperforated cardboard (72%). Mortality rates for larvae in perforated cardboard were intermediate (77%). Application volumes used to deliver nematodes slightly enhanced infection rate of larvae in some substrates but not others. In one trial, parasitism of codling moth by the wasp *Mastrus ridibundus* (Ichneumonidae) was negatively correlated with nematode infection of codling moth larvae. Dissections showed that ca. 10% of larvae infected by nematodes had been attacked by the wasp.

Key Words: **entomopathogenic nematodes; *Steinernema carpocapsae; Cydia pomonella;* codling moth; biological control; orchards.**

INTRODUCTION

Codling moth, *Cydia pomonella* (L.), is the most serious pest of apple production worldwide (Barnes, 1991). More insecticide applications are directed against it than against any of the other insects attacking pome fruits in the western United States (Beers *et al.,* 1993). Since recent successful implementation of mating disruption (MD) of codling moth with synthetic female sex pheromone (Vickers and Rothschild, 1991; Howell *et al.,* 1992), organophosphate insecticide use has declined in many Washington orchards (Calkins, 1998). Unfortunately, MD may not provide adequate control in orchards with uneven topography, at high codling moth densities, and at the beginning of implementation (Calkins, 1998). Hence, applications of azinphosmethyl are often used to supplement MD. Ongoing reevaluation of pesticide registrations mandated by the United States Food Quality Protection Act of 1996 suggests that continued reliance on several organophosphate insecticides, even for supplemental control, is shortsighted. Effective alternatives to these organophosphates would be valuable for both organic and conventional pome-fruit growers. Here we explore the utility of the nematode *Steinernema carpocapsae* (Weiser) as a supplemental control of cocooned codling moth larvae that may complement MD while conserving other naturally occurring control agents in the orchard agroecosystem.

In the Yakima Valley, there are at least two generations of codling moth each year (Newcomer, 1950; Beers *et al.,* 1993). Females, following mating, deposit about 50 eggs individually on leaves and fruits of apple and pear (and other suitable hosts, such as quince and crab apple). Within a few hours of hatching, neonate larvae locate and bore into fruit where they remain and feed until they are mature 5th instar larvae. These larvae exit the fruit and seek cryptic, protected habitats on or below the tree in which to spin their cocoons. Larvae develop through the pupal stage and fly in

[1] To whom correspondence should be addressed. Fax: (509) 454-5646. E-mail: unruh@yarl.ars.usda.gov.

1049-9644/00 $35.00

about 2 weeks under long-day summer conditions but diapause as larvae under the short days of fall (Audemard, 1991). Mature larvae from the second generation usually spin their cocoons after mid-August and do not begin to pupate until late March in the Yakima Valley (Newcomer, 1950; T. R. Unruh, unpublished).

Like soil environments, cryptic habitats such as crevices in wood are thought to be favorable for entomopathogenic nematodes (Begley, 1990; Kaya and Gaugler, 1993; Gaugler *et al.*, 1997). *S. carpocapsae* was originally isolated from codling moth in the Czech Republic and the eastern United States (Weiser, 1955; Dutky and Hough, 1955) and has subsequently been reported from codling moth in the western United States, Mexico, and Poland [summarized by Poinar (1991)]. Our recent laboratory and greenhouse studies demonstrate that mature 5th instar codling moth larvae, in their cocoons, are very susceptible to *S. carpocapsae* and other nematodes (Lacey and Unruh, 1998; Lacey and Chauvin, 1999). Also, previous field trials using *S. carpocapsae* to control cocooned codling moth larvae in natural and artificial substrates demonstrated some potential of this approach (Dutky, 1959, 1974; Kaya *et al.*, 1984; Sledzevskaya, 1987; Nachtigall and Dickler, 1992). Here we present the results of several field experiments that expand on these earlier studies. We examine the importance of available water in enhancing efficacy and compare approaches to measuring infection rates using sentinel cocooned hosts. This is the first such study conducted in the irrigated, cool-desert, pome-fruit production areas of the Pacific Northwest, United States.

MATERIALS AND METHODS

Nematodes. *S. carpocapsae* strains (All strain in 1997, Sal strain in 1998) were purchased from Integrated BioControl Systems, Inc. (Aurora, IN) as infective juveniles (IJs) stored on moist sponge. These were stored at 10°C for up to 2 weeks prior to use. Eight to 24 h prior to use in field experiments, nematodes were suspended in water and quantified using standard procedures (Kaya and Stock, 1997). Stocks were diluted to 10,000–20,000 IJs/ml and suspensions were kept cool (15°C) and aerated with an aquarium bubbler until diluted to the final concentration(s) in the field.

Orchards. A commercial organic orchard in Moxee, Washington consisting of 4.5 ha of ca. 20-year-old var. "Red Delicious" apples with ca. 20% of fruit infestation by larvae was used for Trials 1 and 2 in mid-September 1997. Planting density at Moxee was 540 trees/ha. A third trial was conducted in August 1998 in a USDA-ARS research orchard located 12 km east of Moxee. The research orchard consisted of 1.7 ha of 6-year-old var. "Fuji" apple trees planted at 1000 trees/ha. Weather data were downloaded from the Washington State Public Agricultural Weather System station in Moxee.

Host deployment and mortality assessment. Tree bands, sentinel strips, and sentinel logs were used to sample codling moth and to assess mortality rates in one or more of the field trials (Lacey *et al.*, 2000). Tree bands consisted of two layers of 3-cm-wide, single-faced, cardboard (B flute; Weyerhauser, Tacoma, WA). These bands were wrapped around the tree trunk such that the smooth paper face of one layer was against the trunk and the smooth paper face of the other layer was facing out. These double layers were affixed with two 7 mm staples ca. 30 cm above ground. Mature codling moth larvae readily use artificial substrates such as burlap sacking and cardboard bands in which to spin their cocoons (Newcomer, 1950). Trap bands were deployed in the field 1 month before nematode sprays to allow time for bands to become infested.

Sentinel strips consisted of cardboard strips infested with diapausing codling moth. Diapause-destined codling moth larvae were reared in the laboratory on thinning apples under short photophase (8:16 h L:D). Upon exiting from fruit, larvae spun cocoons in 1.9-cm-wide cardboard strips (double-faced, B flute; Weyerhauser). Infested strips were cut to a length to contain ca. 20 larvae (15–25 cm) for use in orchard trials. In 1998, half of the sentinel strips were perforated (three rows of ca. 0.25-mm-diameter perforations, 5/cm/row) before being infested with larvae as described in Lacey and Unruh (1998). In 1997 trials, no perforated sentinel strips were used.

Sentinel logs were also provided as a supplemental method to assess mortality in the 1998 trial. Logs consisted of ca. 30- to 45-cm lengths of apple wood (scaffold branches), 25–40 cm in circumference. The logs were heat-treated at 66°C for 24 h to eliminate resident insects and reduce fungal growth. Five to six lateral grooves (5 mm wide, 4–6 mm deep, 5–10 cm long) about 1 cm apart were cut into opposite sides of each log to provide cocooning sites for codling moth larvae. Logs were infested with diapause-destined mature 5th instar larvae, reared as described above, for use in the field. More than 95% of the larvae spun their cocoons in the cut grooves and only these larvae were examined during mortality assessments. With the exception of the cut grooves, these logs were similar to those used by Lacey and Unruh (1998).

Quality control. In all three trials described below, 100-ml samples of each treatment were sprayed into collection flasks in the first, middle, and last replicate plots and nematodes were checked for quality. Briefly, the number of living nematodes was counted and they were categorized as active or inactive. Also, a discriminating-dose (LC_{90}) bioassay was conducted on codling moth in triplicate as outlined in detail in Lacey and Unruh (1998). For all studies reported here, nematode

bioassays were always within 85–95% mortality and there was no pattern of increasing or decreasing activity during the experiments.

Trial 1. In one area of the Moxee orchard, trees bearing >100 fruit and exhibiting codling moth damage were banded with cardboard during the first and second weeks of August 1997. During early September, the banded trees were organized into 21 plots of four adjacent banded trees. At least two unsprayed buffer trees separated other such plots within the same tree row, and no plots were directly adjacent in the neighboring tree rows. On 15 September, nonperforated sentinel strips were added to the two center trees in each plot; one strip was stapled vertically to the trunk just above the cardboard band and another strip was placed on the ground within 20 cm of the tree base. At 1800 h on 15 September, orchard irrigation (below tree sprinklers) was turned on and left running until 0800 h on 16 September.

Nematode suspensions were applied with a customized air-blast sprayer (Victair, Hauff and Sons, Inc., Yakima, WA) in Trial 1. It was calibrated to deliver 3.76 liters/tree for each four-tree plot through three nozzles (No. 6; Teejet, Boise, ID; operated at 4.0 kg/cm^2). Nozzles were arranged on a single boom to spread spray evenly from 0.5 m out from the tree base to 1.75 m height into the canopy. Three nematode treatments were used: 5 million IJs/tree, 0.5 million IJs/tree, and a water-only control. These three treatments were randomly assigned to three plots in each of seven blocks. Both sides of the tree row (=plot) were sprayed, resulting in 7.52 liters/tree area. Spraying began at 1430 h and was completed by 1700 h of 16 September.

Trial 2. This experiment was conducted on the same day and at the same orchard as Trial 1 but differed from Trial 1 in method of application, time of day, nematode numbers applied, use of a postwetting treatment, and size of plots. As in Trial 1, trees had been banded previously and received ground and tree sentinel strips, and the orchard had been prewetted by overnight irrigation. Nematode suspensions were applied with a hand-held spray gun (Gunjet No. 2; Teejet) attached to an engine-driven, 95-liter sprayer (Rears Manufacturing, Eugene, OR) running at 4.0 kg/cm^2 and set to spray 3.76 liters in ca. 15 s. The operator carefully sprayed in a smooth motion that maintained the nozzle about 0.5 m from substrates and wet the ground from about 0.5 m out from the tree base to 1.75 m up in the tree canopy. One nematode concentration was used (2 million IJs/tree, in 3.8 liters). There were 42 single-tree plots; each sprayed tree was separated by at least one unsprayed buffer tree from others. Six treatment combinations were used: (1) nematodes applied in the morning, (2) nematodes applied in the morning followed by a water misting (=postwetting), (3) a water control applied in the morning followed by postwetting, (4) as in 1 but nematodes applied in the early evening, (5) as in 2 but nematodes applied in the early evening, and (6) as in 3 but water control applied in the early evening. These six treatment combinations were randomly arranged in seven replicate blocks. Morning applications began at 0900 h and were completed by 1030 h on 16 September. Evening applications began at 1700 h and were completed by 1900 h. Roughly 1 h after application of nematode sprays, trees to be postwetted were lightly misted with water using a hand-gun applicator, taking care to minimize runoff of water (and presumably minimizing rinse-off of nematodes).

Trial 3. This experiment was conducted at the Yakima Agricultural Research Laboratory Moxee Research Farm on 29 July 1998 following protocols similar to those used in Trial 2. A 1.5-ha section of orchard was organized into nine plots each consisting of four subplots and each subplot consisting of two treatment and two buffer trees. Each treatment tree received a sentinel log, a perforated sentinel strip, and a nonperforated sentinel strip before 1200 h on 28 July. Logs were secured to trees with wires such that the grooves were ca. 30 cm above ground level and one set of grooves faced the tree and the other set faced into the tree row. This arrangement was intended to cause the inner grooves (=log back) to be less accessible to direct spray and more protected from drying than the set of grooves facing out (=log front). Strips were stapled vertically on the trees on either side of the log. Two wetting treatments were randomly assigned to plots and consisted of four combinations: ±prewetting and ±postwetting. The two treated trees within each plot were randomly assigned a nematode suspension volume of 1.4 liters/tree or 5.6 liters/tree (each tree represented a subplot). IJ concentrations were calibrated so that both volumes delivered 1 million IJs/tree.

Hand-gun application was as described for Trial 2 with each sprayed tree separated by at least one tree and one buffer row from other such treated trees. Because of previous experience with low control mortality, only two control combinations were deployed: those receiving both pre- and postwetting and those receiving no wetting. These were deployed between treatment rows and represent our exception to one buffer tree separating all sprayed trees. These control trees showed mortality less than 5% in all cases and were not used in the subsequent analyses.

Trees were treated with nematode suspensions between 1630 and 1830 h, preceded by a prewetting ½ h before treatment, where applicable. Postwetting, where applicable, began ½ h after nematode applications and was repeated every hour until 2400 h. Using hoses in the orchard and fine-mister nozzles facilitated postwetting. Essentially, we tried to keep trees damp,

such as might occur during a fine rain or might be produced by overhead sprinklers.

Mortality assessment. Bands, sentinel strips, and sentinel logs were collected from the field ca. 24 h after nematode application, returned to the laboratory, and incubated for 4–5 days at 25°C. The insects were carefully dissected from the substrates and classified as healthy, nematode-infected, wasp-parasitized larvae (see Results), pupae, missing (empty cocoons), or dead due to unknown factors. The proportion of codling moth killed by nematode infection was calculated as the number of infected larvae divided by the sum of nematode-infected, parasitized, and healthy larvae. Pupae and unknown mortality were excluded from the analyses but they represented less than 1% of the total hosts in bands and ca. 5% of hosts in sentinel strips used in 1997. Inclusion of these did not qualitatively influence results or conclusions and generally reduced infection rates by less than 2% (unpublished data).

Statistical analyses. Proportion mortality was arcsine transformed before ANOVA. In Trial 1, mortality was analyzed by ANOVA with nematode application rate as a single treatment term. Post hoc mean comparisons of treatment effects used *t* tests produced by the maximum likelihood algorithm of the LSMEANS-TDIFF statement of PROC GLM (SAS, 1996) because empty cells arose from trap bands with no larvae. Analyses were conducted separately for each monitoring method (tree bands, tree sentinels, and ground sentinels). In Trial 2, mortality was analyzed by a one-way ANOVA with the main treatment variable consisting of the proxy variable consisting of six treatments described above. The data were reanalyzed excluding control (=no nematodes) treatments as a two by two factorial ANOVA with timing of application and postwetting treatment effects. In Trial 3, mortality was analyzed by a split-plot, repeated-measures ANOVA with prewetting and postwetting representing main plot effects and volume applied as the split-plot effect, nested within the main plot interaction term. The four substrates containing cocoons represented the repeated measure and were perforated sentinels, nonperforated sentinels, the hidden side of logs (facing the tree), and the exposed side of logs (facing direct spray). The wetting treatments and blocking effects were tested over their interaction, whereas volume applied, interactions between volume and main plot effects, and the three-way main-plot interaction were tested over the residual error (Winer, 1971). Mortality in each substrate was analyzed separately and combined as repeated measures. Interaction between substrate type and treatment effects was also examined statistically in the repeated-measures ANOVA (PROC GLM; SAS, 1996) and graphically.

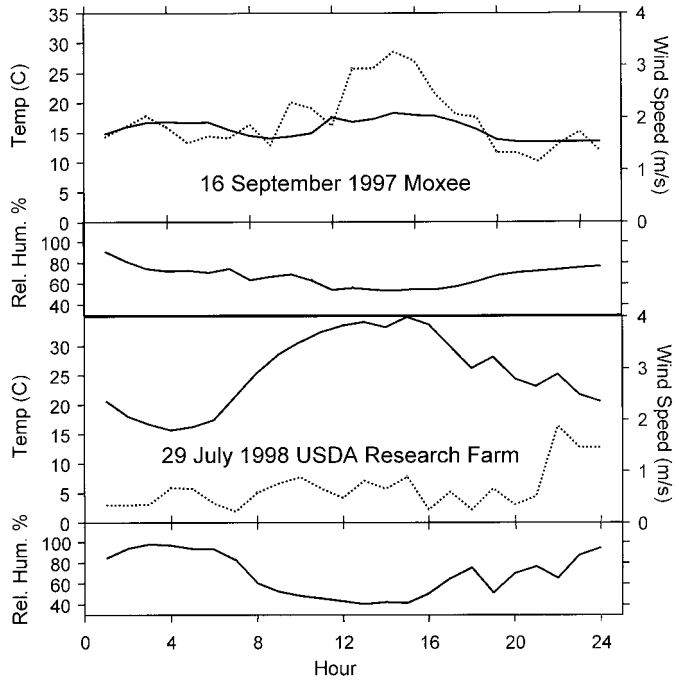

FIG. 1. Temperature, relative humidity, and wind speed (dotted lines) profiles were taken from the Public Agricultural Weather System station in Moxee for days during which experiments were conducted at the Moxee Orchard in September 1997 and the USDA-ARS Research Farm in July 1998.

RESULTS

Temperatures and wind conditions differed as expected between Trials 1 and 2 conducted in autumn of 1997 and Trial 3 conducted in midsummer of 1998, as summarized in Fig. 1. Most notable were temperatures at or below 18°C and wind speeds above 2 m/s in 1997. In summer of 1998, temperature was 25°C or above and wind speeds were less than 2 m/s and often less than 1 m/s.

Trial 1. Standard-speed sprayer applications of 5 or 0.5 million IJs/tree resulted in >30% mortality in tree band substrates (Table 1). Control mortality by nematodes was ca. 3%. Parasitism by *Mastrus ridibundus* Horstmann (Ichneumonidae), an idiobiont, gregarious, external parasitoid of cocooned codling moth larvae (Unruh, 1998) was also observed in tree bands and is reported in Table 1.

Parasitism by *M. ridibundus* in the trap bands was noticed at the outset of scoring and dissection of the bands and caused us to check our ability to discriminate between wasp-parasitized and nematode-infected larvae. Wasp parasitism was identified by the presence of wasp eggs or larvae in the cocoon and by the sclerotized spots on the host cuticle caused by wasp probing or feeding. Also, a yellow-pink color of the host persists until the host cuticle appears loosened and floating on a layer of clear fluid above the inner tissues. Nema-

TABLE 1

Percentage Infection by *Steinernema carpocapsae* and Percentage Parasitism by the Parasitic Wasp *Mastrus ridibundus* of *Cydia pomonella* in Trap Bands Treated with Nematodes by Speed-Sprayer or Hand-Gun Applications

Treatment	*n*	Nematode infected[a]	SE	Wasp parasitized[a]	SE
		Speed sprayer—Trial 1			
5 Million	7	34.6 a	12	6.5 a	2
0.5 Million	7	32.6 a	7	9.7 a	4
Control	7	2.7 b	2	18.2 a	7
		Hand-gun[b]—2 million IJs/tree—Trial 2			
AM no wetting	6	45.4 b	7	12.4 a	1
PM no wetting	7	78.3 a	6	1.8 a b	2
AM wetting	7	72.5 a	8	0.0 b	0
PM wetting	7	69.5 a	12	0.0 b	0
AM control	6	1.3 c	1	4.2 a b	4
PM control	6	0.0 c	0	0.0 b	0

Note. n, number of plots; SE, standard error.

[a] Means in same trial and column followed by the same letter do not differ significantly [corrected *t* test, $P < 0.05$; LSMEANS (SAS, 1996)].

[b] Mean separations based on ANOVA combining time of day and nematode treatments into a single proxy variable. See Results for details.

tode-infected larvae are moribund, shrunken, and flaccid, but the cuticle does not separate from the supporting tissues, and they become tan to brown-gray.

Often, immature wasp stages were displaced or lost in the process of removing codling moth larvae from their cocoon, and differentiation between parasitism by the wasp and parasitism by the nematode was based on color characteristics and, in some cases, dissection. Thirty-four of 37 larvae categorized as nematode infected, based on visual symptoms, contained abundant nematodes of mixed sizes when they were dissected, whereas 3 contained no nematodes. Four of these nematode-infected larvae also had sclerotized spots characteristic of probing by *Mastrus*. Dissection of 13 larvae categorized as attacked by wasps based on color and probing wounds but with no wasp eggs or larvae proved to be uninfected by nematodes (supporting color-based visual characterizations). When 124 additional larvae categorized as nematode infected, based on visual symptoms, were inspected, 15 showed probing wounds.

Nematode treatments were significant in the ANOVA ($F = 7.0$; $df = 2,17$; $P = 0.006$) for infection rates in tree bands. Nematode infection rates were higher in both the 5 million (35%; $t = 2.85$; $P = 0.02$) and the 0.5 million (33%; $t = 2.26$; $P = 0.05$) IJs/tree treatments than in the water-treated control (2.7%) (Table 1). In contrast to tree bands, mortality in nonperforated sentinel strips was low: 8, 5, and 2% for 5 million, 0.5 million, and 0 IJs/tree treatments. These small differences among treatment levels in infection rates for larvae in nonperforated sentinel strips on both the tree and the ground were not significant ($P > 0.5$). We consider the results from nonperforated sentinel strips to be aberrant in this trial and do not use them in further analyses or discussion.

Differences in *Mastrus* parasitism rates in trap bands (but not in nonperforated sentinels) among the nematode treatments was also statistically significant but in an inverse order relative to that of nematode infection rates (Table 1). A similar inverse relationship between wasp parasitism and nematode infection was seen among individual bands (data not shown). We reserve further analyses of the wasp data for studies designed specifically to address the nematode–wasp interaction.

Trial 2. Nematodes applied by hand-gun at 2 million IJs/tree produced 45 to 78% mortality of codling moth in tree bands (Table 1). When the postwetting by time of nematode application treatments and the control (=no nematodes) treatments were combined into a single proxy treatment variable with six levels as described under Materials and Methods, the treatments were highly significant (Table 1; $F = 24.7$; $df = 5,26$; $P = 0.0001$). However, when control treatments (no nematodes) were excluded and time of nematode applications and ±postwetting were analyzed as a two by two factorial ANOVA, main treatment effects were insignificant (time: $F = 1.35$; $df = 1,26$; $P = 0.258$; postwetting: $F = 0.40$; $df = 1,26$; $P = 0.533$). The interaction of these crossed effects was significant ($F = 4.38$; $df = 1,26$; $P = 0.048$), due substantially to lower infection rates in the morning application with no postwetting treatment combination (Table 1).

Again, codling moth larvae exposed in nonperforated sentinel strips showed much reduced mortality due to nematodes (3.5 to 10.7%), produced a nonsignificant treatment term ($P > 0.5$) in the ANOVA, and are not examined further.

As in Trial 1, parasitism by *M. ridibundus* was ob-

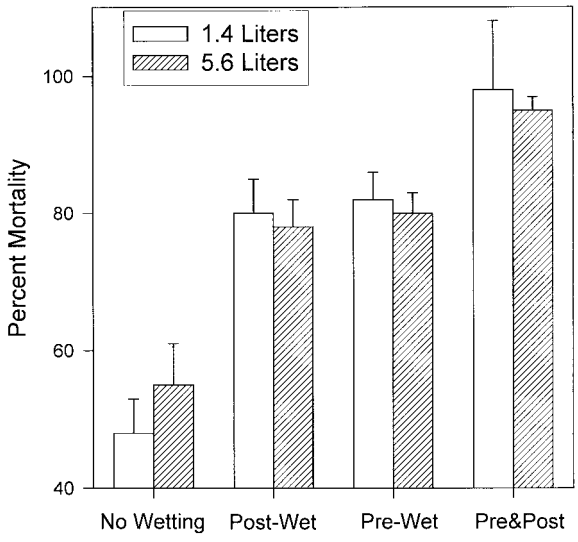

FIG. 2. Average codling moth mortality caused by treatment of 1 million infective juvenile *Steinernema carpocapsae* as influenced by prewetting and postwetting treatments. Data are pooled over volumes in which nematodes were applied and sentinel substrates used to estimate mortality.

served but it was more sporadic and less obviously related to nematode infection rates (Table 1). Of 454 larvae categorized as nematode infected, none had sclerotized wounds characteristic of *Mastrus* probing or feeding. Of 23 larvae categorized as nematode infected, all were infected upon dissection. One of these contained only a single mature adult nematode.

Trial 3. Application of 2 million IJs/tree produced between 40 and 100% mortality of codling moth, depending on pre- and postwetting treatments, volume applied, and substrate type (Figs. 2 and 3). No parasitism by *M. ridibundus* was observed in this experiment. Control mortality averaged <2% and in only one case approached 5%. Hence, we did not correct data for control mortality.

Both the pre- and the postwetting factors were highly significant terms in the main-plots portion of the repeated-measures ANOVA (prewetting: $F = 38.42$; $df = 1,24$; $P < 0.0001$; postwetting: $F = 35.54$; $df = 1,24$; $P < 0.0001$), whereas their interaction and the blocking term were not ($P > 0.05$). Volume in which nematodes were applied was insignificant, as were the interactions of this effect with the pre- and the postwetting main-plot effects ($P > 0.4$ in all cases). These tests are consistent with the pattern of increasing mortality with increasing wetting evident in Fig. 2.

Method of mortality assessment (=substrate housing sentinel larvae) was highly significant ($F = 7.03$; $df = 3,72$; $P = 0.0008$ [Greenhouse–Geisser (G–G) correction]), as was its interactions with the postwetting effect ($F = 6.44$; $df = 3,72$; $P = 0.0014$ [G–G]), with the volume applied ($F = 5.6$; $df = 3,72$; $P = 0.0033$ [G–G]), and with the volume by prewetting interaction ($F = 7.67$; $df = 1,24$; $P = 0.0005$ [G–G]). Mean infection rates from the back of logs (72.6 ± 3.7%) and from nonperforated sentinels (72.0 ± 3.6%) were less than the rate from the front of logs (85.7 ± 2.7%). The mean infection rate from perforated sentinels (77.1 ± 3.7%) was intermediate and not different from either group. The interaction between method of mortality assessment and wetting treatments are explored in Fig. 3. Specifically, mortality of larvae in cardboard sentinels increased more with postwetting (steeper lines in Fig. 3) than did larvae in logs. Prewetting is shown to be especially important (differences between line heights) in logs where larvae were facing the tree trunk (log back in Fig. 3).

Not shown in the figures are the effects of volume in which nematodes were applied. When no pre- or postwetting was applied, larvae in logs show ca. 20% higher mortality when nematodes were applied in high volume compared to low volume. Mortality of larvae in cardboard substrates did not respond significantly to volume of application. When prewetting was used, high-volume applications of nematodes again enhanced mortality in log backs by ca. 20% but not in log fronts nor in cardboard sentinels.

The repeated-measures experimental design and the ANOVA model provided increased power for the repeated terms (volume applied and substrate types) because of inherent positive correlations among repeated or split-plot measurements (Horton *et al.*, 1991). This may occur at the expense of reduced power for the main-plot effects (pre- and postwetting) (Winer, 1971). Despite this bias in power, wetting treatments remained statistically more important than substrate

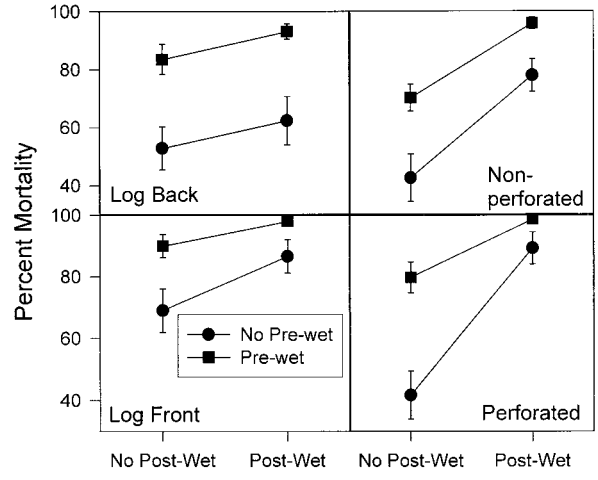

FIG. 3. Plots depicting the interaction among (±) pre- and postwetting treatments on mortality of cocooned codling moth larvae among the four sentinel substrates used to measure mortality (back of log, front of log, nonperforated cardboard strip, perforated cardboard strip). All larvae were treated with 1 million infective juveniles of *Steinernema carpocapsae* but data are pooled over two different volumes of water used in applications (see text).

and especially volume applied. The latter was only important in comparisons among substrates.

DISCUSSION

We conclude that visual symptoms of nematode-induced mortality were accurate indicators of attempted infection by *S. carpocapsae* and that successful development of nematode populations within hosts would occur in at least 90% of those hosts attacked. Further, we found that parasitism by *M. ridibundus* could be separated from mortality caused by nematode infection by visual examination and did not confound our results, even though 10% of the larvae that were nematode infected in Trial 1 also showed symptoms of attack by the wasp. In fact, the simplest explanation for the observed pattern is that some recently parasitized hosts subsequently were infected by nematodes.

The three field trials cannot be compared statistically because they occurred in different seasons, used different nematode isolates, or depended on different substrates for mortality assessments. Despite these differences, the following conclusions appear to be robust: nematode infection was higher in warmer summer conditions, prewetting and postwetting usually enhanced nematode infection rates, the substrates hosting cocooned larvae both affected overall nematode-induced mortality rates and responded differently to pre- and postwetting (Fig. 3), and nematode infection rates were not strongly influenced by the volume in which nematodes were applied, based on the two volumes tested. We suggest that three factors are responsible for differences among the specific experiments and differences seen between treatments within Trials 2 and 3: temperature during infection period, duration of a semiaquatic environment, and method of application.

Temperature during infection period. Nematode species display a unimodal profile that characterizes infection activity by temperature; the sharpness of the activity–temperature curve often differs significantly among species or strains (Grewal *et al.,* 1994; Lacey and Unruh, 1998). We believe that maximum mortality rates observed in the fall of 1997 were lower than those in the summer of 1998 because of the combined effects of lower temperature and higher wind speed during the 6 to 8 h after nematode application in 1997 (Fig. 1). Higher wind speeds will shorten drying time and further lower temperature through evaporative cooling. Average maximum temperatures (1946–1999) for Yakima for July and August is 30.6°C, which is at the high end of the optimal range. Infection by *S. carpocapsae* at 30°C was equal to that observed at 25°C and more than twice that observed at 35°C in laboratory studies (Lacey and Unruh, 1998). This suggests that applications should be applied in the summer on days when temperatures are moderate for central Washington. Dutky (1959, 1974) also reported significant infection rates of cocooned codling moth larvae on trees following treatment with *S. carpocapsae* in the summer.

However, phenology of the nondiapausing summer generation of codling moth is asynchronous enough that cocooned larvae are unlikely to represent a majority share of the stages present in the orchard on any given summer day. Hence, multiple nematode applications are likely to be required to control the summer cohort. In contrast, timing sprays either in autumn or in early spring, when codling moths have accumulated as cocooned larvae in diapause, would target all living codling moths in the orchard.

Average temperatures in central Washington (September–October in Yakima:11–17.5°C) suggest that warm days in autumn should be chosen for nematode applications and that a more cold-adapted nematode strain or species would be useful. Several isolates of *Heterorhabditis* spp., *S. feltiae* (Filipjev) (=*S. bibionis*), and *S. kraussei* (Steiner) are infective at temperatures below 10°C (Griffin and Downes, 1991; Hague *et al.,* 1991; Wright, 1992; Grewal *et al.,* 1996; Mracek *et al.,* 1997). Laboratory studies and field trials targeting fall and spring spray timings with some of these more temperature-resilient species are ongoing.

Duration of a semiaquatic environment. Trials 2 and 3 demonstrate that maintaining available water during the infection phase is critical in maximizing nematode efficacy. We show that both logs and perforated cardboard sentinels, if both pre- and postwetted, allow nearly 100% nematode-induced mortality of cocooned larvae. However, if treated substrates were dry before application and were allowed to dry immediately after application, infection rates were halved. Increased infection rates with postwetting are consistent with our laboratory studies, where highest infections were achieved when humidity was maintained near 100% for 8 h or longer (Lacey and Unruh, 1998). In contrast, nematode infection for codling moth cocoons in grooves in logs facing the tree trunk (a simulated bark crevice) increased more with prewetting than with postwetting. Prewetting probably enhanced penetration and reduced hydrophobicity of these hidden spaces. Unlike Kaya *et al.* (1984), we did not use wetting agents in formulating our nematodes and the value of their use to enhance penetration into bark cracks and crevices bears further investigation.

Higher volume also enhanced infection of larvae in the exposed side of dry logs. Higher application volume may have been beneficial in logs because they may be more easily wetted or may simply blot up more water and retain it longer than paper sentinels. These results suggest that wetting may consist of two components: initial wetting of hydrophobic or hidden surfaces

(=penetration) and long-term maintenance of a moist environment.

Although some entomopathogenic nematodes have mechanisms for surviving dehydration (Simons and Poinar, 1973; Womersley, 1990; Glazer, 1996; Menti *et al.*, 1997), nematode persistence even for short periods can be severely curtailed when moisture and humidity are rapidly reduced (Womersley, 1990). Two approaches to prevent desiccation of nematodes and enhance their activity in the orchard environment have been addressed. First, cardboard banding was covered with a slow-drying, absorbent material such as sheepskin (chamois) or sponge to enhance water retention (Kaya *et al.*, 1984; Nachtigall and Dickler, 1992). Also, Kaya *et al.* (1984) covered bands with aluminum flashing to prevent bird damage and this likely reduced evaporation rates. Codling moth larvae spin cocoons in many cryptic sites in the orchard, both on and off the trees (Geier, 1963; Newcomer, 1950; Higbee *et al.*, 1999, T. R. Unruh, unpublished). We doubt that a large enough proportion of codling moth larvae choose trap bands over other available cocooning sites to make banding (with or without sponges) an economical enhancement to nematode treatments.

Formulation of nematodes with humectants and antidesiccants represents a second method to extend the presence of free water and thereby the activity of infective juveniles in exposed habitats (e.g., Webster, 1973; Glazer, 1992). Furthermore, spraying nematodes with humectants throughout the orchard, including the main branches, the ground, and the tree canopy, may overcome the diversity of cocooning sites. To date, humectants have not been adequately tested for use in temperate orchard environments.

An economical alternative to banding or using humectants is to apply nematodes in rainy weather. Unfortunately, seasonal rainfall may be unpredictable and is likely to covary with temperature. Kaya *et al.* (1984) saw highest infection rates of codling moth larvae following application of *S. carpocapsae* in February in California, when temperatures were just below optimal and rain was common. Under eastern Washington weather patterns, temperatures of summer are close to ideal for most entomopathogenic nematodes but rainfall is more likely to occur in late fall and early spring.

Method of application. The hand-gun applications of nematodes used in Trials 2 and 3 were likely to have deposited IJs in closer proximity to each host larva than did the conventional sprayer applications used in Trial 1, despite corrections for crown-row volume used in the latter trial. Nematode movement is unlikely to exceed 1 cm and is probably much less (Lewis *et al.*, 1991). Hence, delivery of nematodes directly onto cocoons is imperative. Wetting agents may enhance penetration, as discussed above, and thereby enhance distribution. Still, angle of spray application is also likely to be important and modifications of conventional spray equipment for nematode applications in orchards warrants further research.

Finally, we close with a caveat. Estimates of efficacy of *S. carpocapsae* are necessarily based on sentinel or trapped host larvae because of the difficulty of locating and sampling the diversity of cryptic microhabitats which codling moth use for cocooning. Each of the substrates that we used to expose host larvae has positive and negative characteristics but no single substrate is likely to capture the characteristics of the array of habitats that codling moth larvae use in an orchard. It remains possible, if not likely, that some natural habitats in orchards are refugia from nematode infection for codling moth. We still need a method to monitor host infection rates (or other mortality processes) that accurately reflects infection rates in larvae that have freely chosen cocooning sites in the orchard. Ultimately, only whole-orchard or large-plot trials will demonstrate unequivocally the value of nematodes for the control of naturally occurring codling moth populations.

ACKNOWLEDGMENTS

We thank Heather Headrick, Rick Chauvin, Jeff Upton, Martha Marquez, Ivan Campos, Mike Clinton, Gerald Gefre, and Laura Willett for technical support. We are grateful to David Shapiro and James Cate for furnishing cultures of the nematodes and to Harry Kaya and David Horton for constructive comments on the manuscript. David Horton also provided invaluable statistical advice. This research was supported in part through a grant from the Washington Tree Fruit Commission.

REFERENCES

Audemard, H. 1991. Population dynamics of the codling moth. *In* "Tortricid Pests: Their Biology, Natural Enemies and Control" (L. P. S. van der Geest and H. H. Evenhuis, Eds.). pp. 329–338, Elsevier, Amsterdam.

Barnes, M. M. 1991. Tortricids in pome and stone fruits, codling moth occurrence, host race formation, and damage. *In* "Tortricid Pests: Their Biology, Natural Enemies and Control" (L. P. S. van der Geest and H. H. Evenhuis, Eds.), pp. 313–327. Elsevier, Amsterdam.

Beers, E. H., Brunner, J. F., Willett, M. J., and Warner, G. M. (Eds.). 1993. "Orchard Pest Management: A Resource Book for the Pacific Northwest." Good Fruit Grower, Yakima, WA.

Begley, J. W. 1990. Efficacy against insects in habitats other than soil. *In* "Entomopathogenic Nematodes in Biological Control" (R. Gaugler and H. K. Kaya, Eds.), pp. 215–231. CRC Press, Boca Raton, FL.

Calkins, C. O. 1998. Review of the codling moth areawide suppression program in the Western United States. *J. Agric. Entomol.* **15,** 327–333.

Dutky, S. R. 1959. Insect microbiology. *Adv. Appl. Microbiol.* **1,** 175–200.

Dutky, S. R. 1974. Nematode parasites. *In* "Proceedings Summer Institute on Biological Control of Plants, Insects and Diseases"

(F. G. Maxwell and F. A. Harris, Eds.), pp. 576–590. Univ. of Mississippi Press, Jackson.

Dutky, S. R., and Hough, W. S. 1955. Note on parasitic nematode from codling moth larvae, *Carpocapsa pomonella*. *Proc. Entomol. Soc. Wash.* **57,** 244.

Gaugler, R., Lewis, E., and Stuart, R. J. 1997. Ecology in the service of biological control: The case of entomopathogenic nematodes. *Oecologia* **109,** 483–489.

Geier, P. W. 1963. The life history of the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), in the Australian Capital Territory. *Aust. J. Zool.* **11,** 323–367.

Glazer, I. 1992. Survival and efficacy of *Steinernema carpocapsae* in an exposed environment. *Biocontrol Sci. Technol.* **2,** 101–107.

Glazer, I. 1996. Survival mechanisms of entomopathogenic nematodes. *Biocontrol. Sci. Technol.* **6,** 373–378.

Grewal, P. S., Gaugler, R., and Wang, Y. 1996. Enhanced cold tolerance of the entomopathogenic nematode *Steinernema feltiae* through genetic selection. *Ann. Appl. Biol.* **129,** 335–341.

Grewal, P. S., Selvan, S., and Gaugler, R. 1994. Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. *J. Thermal Biol.* **19,** 245–253.

Griffin, C. T., and Downes, M. J. 1991. Low temperature activity in *Heterorhabditis* sp. (Nematoda: Heterorhabditidae). *Nematologica* **37,** 83–91.

Hague, N. G. M., Haukeland, M. S., and Otto, A. A. 1991. Effects of temperature on the efficacy of steinernematid nematodes. *Med. Fac. Landbouww. Rijksuniv. Gent.* **56,** 1245–1251.

Higbee, B. S., Calkins, C. O., and Temple, C. 1999. Larval infestation of harvest bins by codling moth. *Good Fruit Grower* **March 15,** 26–31.

Horton, D. R., Chapman, P. L., and Capinera, J. L. 1991. Detecting local adaptation in phytophagous insects using repeated measures designs. *Environ. Entomol.* **20,** 410–418.

Howell, J. F., Knight, A. L., Unruh, T. R., Brown, D. F., Krysan, J. L., Sell, C. R., and Kirsch, P. A. 1992. Control of codling moth in apple and pear with sex pheromone-mediated mating disruption. *J. Econ. Entomol.* **85,** 918–925.

Kaya, H. K., and Gaugler, R. 1993. Entomopathogenic nematodes. *Annu. Rev. Entomol.* **38,** 181–206.

Kaya, H. K., and Stock, S. P. 1997. Techniques in insect nematology. *In* "Manual of Techniques in Insect Pathology" (L. A. Lacey, Ed.), pp. 281–324. Academic Press, London.

Kaya, H. K., Joos, J. L., Falcon, L. A., and Berlowitz, A. 1984. Suppression of the codling moth (Lepidoptera: Olethreutidae) with the entomogenous nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae). *J. Econ. Entomol.* **77,** 1240–1244.

Lacey, L. A., and Chauvin, R. L. 1999. Entomopathogenic nematodes for control of diapausing codling moth in fruit bins. *J. Econ. Entomol.* **92,** 104–109.

Lacey, L. A., and Unruh, T. R. 1998. Entomopathogenic nematodes for control of codling moth: Effect of nematode species, dosage, and temperature and humidity under laboratory and simulated field conditions. *Biol. Control* **13,** 190–197.

Lacey, L. A., Knight, A., and Huber, J. 2000. Microbial control of lepidopteran pests of apple orchards. *In* "Field Manual of Techniques in Invertebrate Pathology: Application and Evaluation of Pathogens for Control of Insects and Other Invertebrate Pests" (L. A. Lacey and H. K. Kaya, Eds.) pp. 557–576. Kluwer Academic, Dordrecht.

Lewis, E. E., Gaugler, R., and Harrison, R. 1992. Entomopathogenic nematode host finding: Response to host cues by cruise and ambush foragers. *Parasitology* **105,** 309–315.

Menti, H. D., Wright, J., and Perry, R. N. 1997. Desiccation survival of population of the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis* from Greece and the UK. *J. Helminthol.* **71,** 41–46.

Mracek, Z., Becvar, S., Rezac, P., Kindlmann, P., and Webster, J. M. 1997. Canadian steinernematid (Nematoda) isolates and their infectivity, under cold conditions, to greater wax moth (*Galleria mellonella*) larvae. *Biol. Control* **8,** 160–164.

Nachtigall, G., and Dickler, E. 1992. Experiences with field applications of entomopathogenic nematodes for biological control of cryptic living insects in orchards. *Act. Phytopathol. Entomol. Hung.* **27,** 485–490.

Newcomer, E. J. 1950. "Orchard Insects of the Pacific Northwest and Their Control." USDA Circular 270.

Poinar, G. O., Jr. 1991. Nematode parasites. *In* "Tortricid Pests: Their Biology, Natural Enemies and Control" (L. P. S. van der Geest and H. H. Evenhuis, Eds.), pp. 273–281. Elsevier, Amsterdam.

SAS. 1996. SAS/STAT Users Guide, Version 6.12. SAS Institute Inc. Cary, NC.

Simons, W. R., and Poinar, G. O., Jr. 1973. The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survive extended periods of desiccation. *J. Invertebr. Pathol.* **22,** 228–230.

Sledzevskaya, E. R. 1987. Study of the factors determining the activity of the nematode *Neoaplectana carpocapsae* and its efficacy against orchard insect pests. *In* "Helminths of Insects" (M. D. Sonin, Ed.), pp. 152–155. Amerind Pub. Co., New Delhi, India.

Unruh, T. R. 1998. From Russia with love: New predators and parasites for control of tree fruit insect pests. *Proc. Wash. Hort. Assoc. (1997)* **93,** 42–49.

Vickers, R. A., and Rothschild, G. H. L. 1991. Use of sex pheromones for control of codling moth. *In* "Tortricid Pests: Their Biology, Natural Enemies and Control" (L. P. S. van der Geest and H. H. Evenhuis, Eds.) pp. 339–354. Elsevier, Amsterdam.

Webster, J. M. 1973. Manipulation of environment to facilitate use of nematodes in biocontrol of insects. *Exp. Parasitol.* **33,** 197–206.

Weiser, J. 1955. *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinernematidae) novy cizopasnik housenek obalece jablecneho, *Carpocapsae pomonella* L. *Vestn. Cesk. Zool. Spol.* **19,** 44–52. [in Czech]

Winer, B. J. 1971. "Statistical Principles in Experimental Design." 2nd ed. McGraw–Hill, New York.

Womersley, C. Z. 1990. Dehydration survival and anhydrobiotic potential. *In* "Entomopathogenic Nematodes in Biological Control" (R. Gaugler and H. K. Kaya, Eds.), pp. 117–138, CRC Press, Boca Raton, FL.

Wright, P. J. 1992. Cool temperature reproduction of Steinernematid and heterorhabditid nematodes. *J. Invertebr. Pathol.* **60,** 148–151.